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(88) Date of publication of the international search report:  
25 September 2003

## INTERNATIONAL SEARCH REPORT

International application No.

PCT/US03/04761

## A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : A61K 9/00  
 US CL. : 424/451, 464, 489

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)  
 U.S. : 424/451, 464, 489

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)  
 CAPLUS, MEDLINE, EAST

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A, P	US 6,416,808 B1 (CREA) 09 July 2002 (09.07.2002), see entire document.	1-45
A	US 6,165,475 A (CREA et al.) 26 December 2000 (26.12.2000), see entire document.	1-45
A	US 6,197,308 B1 (CREA et al.) 06 March 2002 (06.03.2001), see entire document.	1-45

 Further documents are listed in the continuation of Box C. See patent family annex.

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document member of the same patent family

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Date of the actual completion of the international search

08 May 2003 (08.05.2003)

Date of mailing of the international search report

14 AUG 2003

Name and mailing address of the ISA/US

Mail Stop PCT, Attn: ISA/US  
 Commissioner for Patents  
 P.O. Box 1450  
 Alexandria, Virginia 22313-1450

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THIS PAGE BLANK (USPTO)

(19) World Intellectual Property Organization  
International Bureau(43) International Publication Date  
21 August 2003 (21.08.2003)

PCT

(10) International Publication Number  
WO 03/068171 A2(51) International Patent Classification<sup>7</sup>:

A61K

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(21) International Application Number: PCT/US03/04761

(22) International Filing Date: 13 February 2003 (13.02.2003)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:  
60/356,847 13 February 2002 (13.02.2002) US

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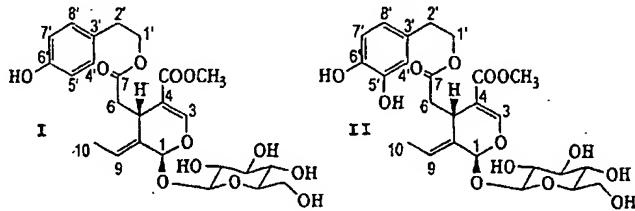
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(81) Designated States (national): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, UZ, VC, VN, YU, ZA, ZM, ZW.

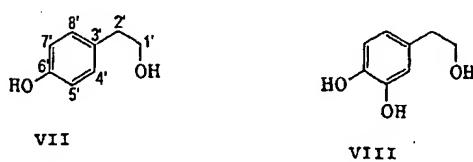
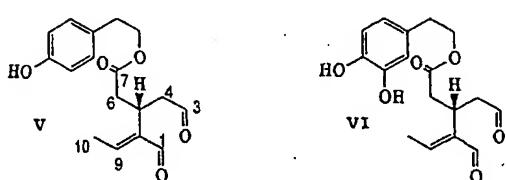
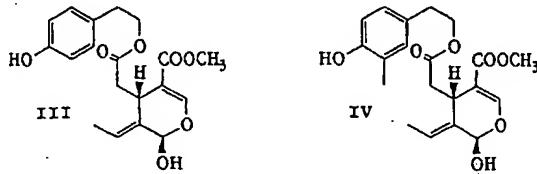
(84) Designated States (regional): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

[Continued on next page]

(54) Title: METHOD AND COMPOSITION FOR TREATMENT OF INFLAMMATION AND AIDS-ASSOCIATED NEUROLOGICAL DISORDERS



(57) Abstract: A method of treating inflammation, an inflammatory condition, or AIDS-associated neurological disorder in a subject in need of such treatment is disclosed. The method includes administering to said subject a pharmaceutically effective amount of substantially purified hydroxytyrosol or a substantially purified mixture of hydroxytyrosol and oleuropein. Also disclosed are compositions for use in practicing the method.



WO 03/068171 A2



**Published:**

— *without international search report and to be republished upon receipt of that report*

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**Method and Composition for Treatment of Inflammation and**  
**AIDS-Associated Neurological Disorders**

**Field of the Invention**

5 The present invention relates to methods for treating AIDS-associated neurological disorders, inflammation and inflammation-associated disorders and to pharmaceutical compositions for use in practicing the method.

**References**

10 Adamson DC, et al. (1999) Rate and severity of HIV-associated dementia (HAD): correlations with Gp41 and iNOS, *Mol. Med.* 5:98-109.

Addis PB, et al (1995) Atherogenic and anti-atherogenic factors in the human diet, *Biochem Soc Symp* 61:259-271.

15 Caruso D, et al. (1999) Effect of virgin olive oil phenolic compounds on in vitro oxidation of human low density lipoproteins, *Nutr Metab Cardiovasc Dis* Jun;9(3):102-107.

Cohen RA, et al (2001) Neurocognitive performance enhanced by highly active antiretroviral therapy in HIV-infected women, *AIDS* 15:341-345.

20 Dal Pan GJ, et al (1994) Clinicopathologic correlations of HIV-1-associated vacuolar myelopathy: an autopsy-based case-control study, *Neurology* 44:2159-2164.

Dore GJ, et al (1999) Changes to AIDS dementia complex in the era of highly active antiretroviral therapy, *AIDS* 13:1249-1253.

25 Ellis RJ, et al (1997) Cerebrospinal fluid human immunodeficiency virus type 1 RNA levels are elevated in neurocognitively impaired individuals with acquired immunodeficiency syndrome, *HIV Neurobehavioral Research Center Group in Ann. Neurol* 42:679-688.

30 Enting RH, et al (2000) Cerebrospinal fluid beta2-microglobulin, monocyte chemotactic protein-1, and soluble tumor necrosis factor alpha receptors before and after treatment with Lamivudine plus zidovudine or stavudine, *J Neuroimmunol* Jan 24;102(2):216-221.

Glass JD, et al (2001) Microglia in HIV-associated neurological diseases, *Microscopy Research and Technique* 54:95-105.

Glass JD, et al (1995) Immunocytochemical quantitation of HIV virus in the brain: correlations with dementia, *Ann Neurol* 38:755-762.

Lipton SA and Gendelman HE (1995) Dementia associated with the acquired immunodeficiency syndrome, *N Engl J Med* 332:934-940.

5 Koutsilieri E, et al (2001) Neurotransmission in HIV associated dementia: a short review, *J Neural Transm* 108:767-775.

Mazur A, et al (1999) Inhibiting effect of procyanidin-rich extracts on LDL oxidation in vitro, *Atherosclerosis* 145:421-422.

Masliah E, et al. (2000) Changes in pathological findings at autopsy in 10 AIDS cases for the last 15 years, *AIDS* 14:69-74.

McArthur JC (1987) Neurologic manifestations of AIDS, *Medicine* 66:407-437.

McGuire D and Marder K (2000) Pharmacologic frontiers in the treatment of AIDS dementia, *J Psychopharmacol* 14(3):251-257.

15 Ng TB, et al (1997) Anti-HIV natural products with special emphasis on HIV reverse transcriptase inhibitors, *Life Sci* 61(10):933-49.

Napoli C and Lerman LO (2001) Involvement of oxidation-sensitive mechanisms in the cardiovascular effects of hypercholesterolemia, *Mayo Clin Proc* 76:619-631.

20 Nottet HS and Gendelman HE (1995) Unraveling the neuroimmune mechanisms for the HI4:281-290V-1-associated cognitive/motor complex, *Immunol Today* Sep;16(9):441-448.

Petito CK, et al (1985) Vacuolar myelopathy pathologically resembling subacute combined degeneration in patients with the acquired immunodeficiency syndrome, *N Engl J Med* 312:874-879.

25 Practico D, et al (1998) Increased F2-isoprostanes in Alzheimer's disease: evidence for enhanced lipid peroxidation in vivo, *FASEB J* 12 (15):1777-1783.

Rice-Evans C (1995) Plant polyphenols: free radical scavengers or chain-breaking antioxidants? *Biochem Soc Symp* 61:103-116.

30 Rimbach G, et al (1999) Methods to assess free radicals and oxidative stress in biological systems, *Arch Tieremahr* 52(3):203-222.

Ryan, LA et al (2001) Peripheral immune activation in HIV-1 associated dementia: elevated plasma levels of soluble CD14 and TNF- $\alpha$  receptor two, *J Infect Dis* 184: 699-709.

Sarkkinen, ES et al (1993) Effects of two low-fat diets, high and low in polyunsaturated fatty acids, on plasma lipid peroxides and serum vitamin E levels in free-living hypercholesterolaemic men, *Eur J Clin Nut* 47:623-630.

5 Shi B, et al (1998) Neuronal apoptosis induced by HIV-1 tat protein and TNF alpha: potentiation of neurotoxicity mediated by oxidative stress and implications for AIDS dementia, *J Neurosci* Nov 15;18(22):9326-34.

Treitinger A, et al (2000) Decreased antioxidant defense in individuals infected by the human immunodeficiency virus, *Eur J Clin Invest* May;30(5):454-459.

10 Visioli F, et al (2000) Olive oils rich in natural catecholic phenols decrease isoprostane excretion in humans, *Biochem Biophys Res Commun* Nov 30;278(3):797-799.

Visioli F and Galli C (2001) Antiatherogenic components of olive oil, *Curr Atheroscler Rep* Jan;3(1):64-67.

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### Background of the Invention

The Acquired Immunodeficiency Syndrome (AIDS), caused by the human immunodeficiency virus type-1 (HIV-1), is one of the ten leading causes of death in the world (Koutsilieri, 2001). According to a June 2000 World Health

20 Organization (WHO) report on global human immunodeficiency virus (HIV)/AIDS pandemic, nearly 35 million adults and 2 million children worldwide are infected with HIV and it is estimated that one third of the adults and more than one half of the children will develop a dementing illness.

25 This virus, transmitted by sexual contact or exposure to infected blood products, has to this point eluded attempts at eradication and continues to spread through human populations in both industrialized and non-industrialized nations. Although modern anti-viral medications may control viral replication and prolong life, there is currently no preventative vaccine and no examples of a cure (Glass, 2001).

30 A variety of neurological syndromes occur throughout the course of HIV infection, affecting the central nervous system, peripheral nervous system, and muscle. The sequelae of HIV infection can be categorized as either related to opportunistic infections of the nervous system, or as direct or indirect effects of the virus itself. Some disorders are manifested early and some late during the

infectious process, and the pathological changes include inflammatory, demyelinating, and degenerative changes. This spectrum of diseases associated with a single virus infection is unique in virology.

5 Neurological disease occurs rarely at the time of initial infection with HIV, even before seroconversion, and prior to the profound immunosuppression of the latter stages of HIV infection. These early manifestations include aseptic meningitis or encephalitis, acute and chronic inflammatory demyelinating polyneuropathies, mononeuritis multiplex associated with peripheral nerve vasculitis, and HIV-associated polymyositis (McArthur, 1987).

10 Oxidative stress has been implicated in a variety of diseases and pathological conditions, including endothelial cell cytotoxicity, cancer, and coronary heart diseases, such as thrombosis and hyperlipemia (Addis, 1995). Recent studies have shown that elevated lipid peroxidation levels (oxidative stress) may play a role in the pathogenesis of Alzheimer's disease which includes 15 a group of neurodegenerative disorders with diverse etiologies, but the same hallmark brain lesions (Pratico, 1998).

20 Clinical studies have established that elevated plasma concentrations of LDL are associated with atherosclerosis, a most prevalent cardiovascular disease and the principle cause of heart attack, stroke and vascular circulation problems (Sarkkinen, 1993). It is believed that a reduction of atherogenic lipid peroxides, which are transported in the LDL fraction of blood serum, can reduce the risk of atherogenesis (Mazur, 1999). Antioxidants limit oxidative modification of LDL and consequently lower plasma concentrations of LDL, thereby acting as anti-25 atherogenic compounds (Sarkkinen, 1993). The oxidation of LDL has been reported as a model for testing the ability of polyphenols to act as antioxidants by breaking the peroxidative cascade described above (Rice-Evans, 1995).

30 A safe, relatively inexpensive, orally administered neuro protectant that reduces the sequelae of cerebral oxidative stress in the setting of HIV-associated brain disease would have great value as an adjunctive therapy. Benefits include those based on evidence regarding the effect of free-radical damage of oxidative stress on key organ systems. They include cerebral antioxidant activity with reduction of ongoing brain injury (Shi 1998; Treitinger 2000); antiviral effects (oxidative stress activates a transcription factor necessary for HIV replication, and phenols are effective in vitro against HIV-1; Ng, 1997); and antiatherosclerotic

cardiovascular benefits. Of note, cardiovascular risk has become a concerning issue among HAART-treated individuals as the incidence of severe hyperlipidemia has increased (Lipodystrophy Syndrome). Oxidative stress inhibits the key enzyme responsible for the transfer and metabolism of cell-derived cholesterol via HDL. Antioxidant therapy increases the capacity of HDL for cholesterol uptake, and could lower cholesterol. In addition, antioxidants have been shown in vitro and in vivo to decrease oxidized LDL, strongly implicated as a mediator of endothelial damage of cardiovascular disease (Caruso 1999, Napoli 2001).

10

### Summary of the Invention

Accordingly, it is an object of the invention to provide, in one aspect, a method for treating an AIDS-associated neurological disorder in a subject in need of such treatment. The method includes administering to the subject a pharmaceutically effective amount of a treatment agent having hydroxytyrosol and oleuropein. In one embodiment, the weight ratio of hydroxytyrosol to oleuropein of between about 1:1 and about 200:1. In another embodiment, the weight ratio is between about 5:1 and about 100:1. In yet another embodiment, the weight ratio of hydroxytyrosol and oleuropein is between about 10:1 and about 50:1.

In a preferred embodiment, the treatment agent is prepared by a process comprising the steps of producing vegetation water from olives, adding acid to the vegetation water in an amount effective to produce a pH between about 1 and about 5, and incubating the acidified vegetation water until at least 75% of oleuropein originally present in the vegetation water has been converted to hydroxytyrosol.

An exemplary disorder for treatment is AIDS dementia. A preferred route of administration includes oral delivery.

In one embodiment, the administering further includes administering a second disease treatment agent. Administering the second treatment agent may be before or after administration of the first treatment agent. Alternatively, administering the second treatment agent is coincident with administering the first treatment agent. Preferably the second treatment agent is an antiretroviral agent.

In another embodiment, the first agent is administered at a dosage of

between about 0.1 mg/kg and 2000 mg/kg per day. Preferably, the first agent is administered at a dosage of between about 0.3 mg/kg and 1 mg/kg per day. Even more preferably, the agent is administered at a dosage of about 0.6 mg/kg per day.

5 In one embodiment, the subject is a human. In another embodiment, the agent is dried to provide a powder extract. In yet another embodiment, the agent is in the form of a tablet, capsule, or pill.

In another aspect, the invention contemplates a method of treating an AIDS-associated neurological disorder in a subject in need of such treatment.

10 The method includes administering to said subject a pharmaceutically effective amount of substantially purified hydroxytyrosol or a substantially purified mixture of hydroxytyrosol and oleuropein.

15 Another aspect of the invention includes a method of treating a subject having an inflammatory condition characterized by a detectable clinical symptom or change in a level of a biochemical marker with respect to the normal range of the marker. The method includes administering to the subject a dose of an olive plant extract treatment agent. In one embodiment, the extract has a weight ratio of hydroxytyrosol to oleuropein of between about 1:1 and about 200:1. In another embodiment, the weight ratio is between about 5:1 and about 100:1. Preferably 20 the weight ratio is between about 10:1 and about 50:1.

The method further includes continuing said administration until there is observed a return of the marker level to the normal range or a desired change in the clinical symptom. The marker or the clinical symptom may be (i) the symptoms and markers in joint pain and swelling in the case of joint inflammation; 25 (ii) elevated levels of C-reactive protein in the case of coronary inflammation; (iii) respiratory distress in the case of bronchial inflammation; and/or (iv) elevated CSF levels of isoprostanes or functional or psychofunctional indicators in the case of neuro-inflammation.

30 In one embodiment, the marker is a cytokine such as tumor necrosis factor- $\alpha$ , interleukin-1, interleukin-6, and/or interleukin-8.

In another embodiment, the marker is corticotrophin, cortisol and/or prolactin.

In one embodiment, the inflammatory condition is selected from the group consisting of rheumatoid arthritis, osteoarthritis, and other inflammatory conditions

involving acute joint inflammation, chronic joint inflammation, or both.

The extract may be prepared by a process that includes (a) producing vegetation water from olives; (b) adding acid to the vegetation water in an amount effective to produce a pH between about 1 and about 5; and (c) incubating the acidified vegetation water until at least 75% of oleuropein originally present in the vegetation water has been converted to hydroxytyrosol.

The administration may include a method selected from the group consisting of oral delivery, intramuscular injection, intravenous injection, transdermal delivery, and/or transmucosal delivery. Preferably the administering includes oral delivery.

In one embodiment of the invention, the administering further comprises administering a second disease treatment agent. The administering of the second treatment agent may be before, after or coincident with administration of the first treatment agent. The second treatment agent includes one or more of the components selected from the group consisting of glucosamine sulfate, chondroitin sulfate, sea cucumber extract, hydrolyzed shark cartilage, collagen II, and methylsulfonylmethane.

In one embodiment of the invention the agent or extract is administered at a dosage of between about 0.1 mg/kg and 2000 mg/kg per day. Preferably the agent or extract is administered at a dosage of between about 0.3 mg/kg and 1 mg/kg per day. More preferably, the agent is administered at a dosage of about 0.6 mg/kg per day.

In one embodiment, the subject is a human.

In another embodiment, the agent is dried to provide a powder extract.

In yet another embodiment, the agent is in the form of a tablet, capsule, or pill. Alternatively, the agent may be in the form of a liquid or liquid drops.

In a broader aspect, the invention provides a method of treating an inflammatory condition in a subject in need of such treatment, comprising administering to said subject a pharmaceutically effective amount of substantially purified hydroxytyrosol or a substantially purified mixture of hydroxytyrosol and oleuropein. In one embodiment, the inflammatory condition is in response to a condition selected from the group consisting of delayed type hypersensitivity reaction, a symptom of psoriasis, an autoimmune disease, organ transplant, pain, fever, and tissue graft rejection. Preferably, the autoimmune disease is selected

from the group consisting of: Reynaud's syndrome, autoimmune thyroiditis, EAE, multiple sclerosis and lupus erythematosus.

In another embodiment, the inflammatory condition is in response to a condition selected from the group consisting of adult respiratory distress syndrome (ARDS), multiple organ injury syndromes secondary to septicemia or trauma, reperfusion injury of myocardial or other tissues, acute glomerulonephritis, reactive arthritis, dermatoses with acute inflammatory components, acute purulent meningitis or other central nervous system inflammatory disorders, thermal injury, hemodialysis, leukapheresis, ulcerative colitis, Crohn's disease, necrotizing enterocolitis, granulocyte transfusion associated syndromes, and cytokine-induced toxicity.

In yet another embodiment, the inflammatory condition results from a condition selected from the group consisting of asthma, psoriasis, skin sunburn, inflammatory pelvic disease, inflammatory bowel disease, urethritis, uritis, 15 senusitis, pneumonitis, encephalitis, meningitis, myocarditis, nephritis, osteomyelitis, myositis, hepatitis, gastritis, enteritis, dermatitis, gingivitis, appendicitis, pancreatitis, cholecystitis and cholangitis.

In still another embodiment, the inflammatory condition is an acute inflammatory reaction. Alternatively, the inflammatory condition is an allergic 20 inflammatory reaction.

These and other objects and features of the invention will be more fully appreciated when the following detailed description of the invention is read in conjunction with the accompanying figure.

25 **Brief Description of Figure**

Figure 1 shows the structures of phenolic compounds and their precursors detected in olive oil: ligstroside (I); oleuropein glucoside (II); aglycone of ligstroside (III); aglycone of oleuropein glucoside (IV); dialdehydic form of ligstroside aglycone lacking a carboxymethyl group (V); dialdehydic form of 30 oleuropein glucoside aglycone lacking a carboxymethyl group (VI); tyrosol (VII); hydroxytyrosol (VIII).

**Detailed Description of the Invention**

I. **Definitions**

Unless otherwise indicated, all terms used herein have the same meaning as they would to one skilled in the art of the present invention. It is to be understood that this invention is not limited to the particular methodology, protocols, and reagents described, as these may vary.

5 The term "effective amount", as used herein, represents an amount of agent necessary to prevent or treat a subject susceptible to or suffering from an AIDS-associated neurological condition or an inflammatory response following administration to such subject. The active compound may be effective over a wide dosage range. It will be understood that the amount of the compound actually 10 administered will be determined by a physician, in light of the relevant circumstances including the condition to be treated the age, weight, and response of the individual patient, the severity of the patient's symptoms, and the chosen route of administration.

15 As used herein, the term "treating" includes prophylaxis of a physical and/or mental condition or amelioration or elimination of the developed physical and/or mental condition once it has been established or alleviation of the characteristic symptoms of such condition.

By "oleuropein" is intended secoiridoid glucoside oleuropein (Structure II in Figure 1).

20 By "tyrosol" is intended 4-hydroxyphenethyl alcohol (Structure VII in Figure 1).

By "hydroxytyrosol" is intended 3, 4-dihydroxyphenethyl alcohol (Structure VIII in the Figure 1).

25 The term "substantially purified", as used herein, refers to a compound or compounds that are removed from their natural environment, isolated or separated, and are at least 60% free, preferably 75% free, more preferably 85% free, even more preferably 90% free, still more preferably 95% free, and most preferably 99% free from other components with which they are naturally associated.

30

## II. Method of the Invention

The invention includes, in one aspect, a method of treating an AIDS-associated neurological disorder. Another aspect of the invention provides a method of treating inflammation or an inflammation-associated disorder. In

practicing the method of the invention, the composition as described below, is preferably formulated as a tablet formulation, is preferably administered orally at a desired dose and dosing schedule.

5 The method of the invention employs phenolic compounds. Hydroxytyrosol and oleuropein are preferred phenols. The phenolic compounds may be synthesized or extracted and/or purified by methods known to those of skill in the art.

#### A. Olive-Derived Phenols

10 Preferably, the phenolic compounds are derived from olives that may be obtained from conventional and commercially available sources such as growers. A number of phenolic compounds are found in olives and olive oil, including relatively apolar, oil soluble phenolic compounds as well as relatively polar, water soluble phenolic compounds. In the context of the present invention both groups are 15 denoted as phenols. Apolar phenols comprise the compounds oleuropein, ligstroside and their aglycons. Polar phenols comprise tyrosol, hydroxytyrosol, caffeic acid and vanillic acid.

20 The olive-derived phenolic compounds employed herein can be prepared by a number of methods known in the art. The olives may be processed by any suitable means to obtain the compositions described. In one embodiment of the invention, the olives are pressed to obtain a mixture including olive oil, vegetation water, and solid by-products. The phenolic compounds may be obtained directly from the mixture, or the mixture may be fractionated and/or purified to obtain the compounds of the invention. The compositions may be fractionated and/or purified 25 by a number of methods known to those of skill in the art. Exemplary methods for fractionation include partitioning with an organic solvent, high pressure liquid chromatography (HPLC), or the use of supercritical fluids.

Exemplary methods for preparing phenolic compositions derived from olives in accordance with the invention may be found in co-owned U.S. Patent Nos. 30 6,165,475, issued Dec. 26, 2000 and 6,197,308, issued March 6, 2001, each of which is expressly incorporated by reference herein. An exemplary method for preparing hydroxytyrosol-rich compositions from olive vegetation water is found in co-owned U.S. Patent Application No. 09/944,744, filed August 31, 2001, which is expressly incorporated by reference herein. Techniques suitable for concentrating

and/or isolating oleuropein from aqueous and aqueous-alcoholic solutions are taught, for example, in U.S. Pat. No. 5,714,150, expressly incorporated herein by reference.

5 A preferred method of obtaining hydroxytyrosol and/or oleuropein is considered below:

### 1. Producing Vegetation Water

Preferably, the vegetation water is obtained from pitted olives. The olives processed according to the method disclosed herein may be pitted by any 10 suitable means. Pits in the olives contain tyrosol which is an undesired component in the vegetation water and which may not be appreciably eliminated by the acid treatment described below. The pits may be separated from the pulp manually or in an automated manner as described below. Preferably, such means should be capable of segregating the pits without breaking them, which 15 might otherwise cause higher concentrations of tyrosol in the vegetation water. In another embodiment, hydroxytyrosol is extracted from vegetation water obtained from olives that have not been pitted.

To produce vegetation water, olive pulp from the olives is first pressed to obtain a liquid-phase mixture including olive oil, vegetation water, and solid by- 20 products. Thereafter, the vegetation water is separated from the rest of the liquid phase mixture and collected. Exemplary methods of obtaining vegetation water are described in the above-referenced, co-owned U.S. Patent Nos. 6,165,475 and 6,197,308, both to R. Crea, each of which are expressly incorporated herein by reference in their entirety.

25 For purposes of commercial production, it may be desirable to automate various aspects of the invention. In this regard, one embodiment contemplates the use of an apparatus as disclosed in U.S. Patent Nos. 4,452,744, 4,522,119 and 4,370,274, each to Finch *et al.*, and each expressly incorporated herein by reference. Briefly, Finch *et al.* teach an apparatus for recovering olive oil from 30 olives. Initially, olives are fed to a pulper that separates the olive pits from the olives to obtain a pitless olive meat. The meat is then taken up by an extraction screw that subjects the meat to an extraction pressure sufficient to withdraw a liquid phase, comprising oil, water and a minor proportion of olive pulp. The liquid phase is collected in a bin and then sent to a clarifying centrifuge that separates

the pulp from the liquid phase to obtain a mixture comprising olive oil and vegetation water. A purifying centrifuge then separates the vegetation water and a small proportion of solid matter from the mixture to obtain an olive oil, substantially free of vegetation water, that is collected in a tank.

5 Additional devices that may be used in practicing the present invention are disclosed in Italian Patent Nos. 1276576 and 1278025, each of which is expressly incorporated herein by reference. As above, these devices can be used to separate the pulp from the pits prior to processing of the crushed olive pulp into oil, water, and solid residues.

10

## 2. Conversion of Oleuropein to Hydroxytyrosol

In one aspect of the invention, the oleuropein contained in the vegetation water is converted to hydroxytyrosol. The pH of the vegetation water may be decreased by the addition of acid (organic or inorganic), and the 15 vegetation water allowed to incubate under conditions which promote hydrolysis of oleuropein to hydroxytyrosol. The sample may then be fractionated to separate hydroxytyrosol from other compounds.

In a preferred embodiment, the added acid is citric acid. The acid is added to the vegetation water to adjust the pH to 1-5, preferably 2-4. Solid citric acid 20 can be added while continuously stirring in an amount of preferably about 25 to 50 pounds of acid per about 1000 liters of vegetation water. The pH of the resulting solution can be monitored, and further addition of acid may be necessary to achieve the desired pH.

The acid may also be an organic or inorganic acid other than citric acid. 25 Exemplary acids which may be used in the present invention include the inorganic substances known as the mineral acids -- sulfuric, nitric, hydrochloric, and phosphoric acids -- and the organic compounds belonging to the carboxylic acid, sulfonic acid, and phenol groups. The addition of acid to the vegetation water serves several purposes: (i) it stabilizes the vegetation water from rapid 30 oxidation by the air/oxygen; (ii) it prevents fermentation (bacteria) of the vegetation water; and (iii) it hydrolyzes the oleuropein, converting it to hydroxytyrosol. In one embodiment, the mixture is allowed to incubate until hydroxytyrosol is 75-90% of the total combination of tyrosol and hydroxytyrosol, and substantially none of the oleuropein in the original mixture remains.

### 3. Purification of Hydroxytyrosol

Following the conversion of oleuropein to hydroxytyrosol, the vegetation water may be fractionated by a number of methods known in the art.

5 Alternatively, vegetation water may be fractionated prior to treatment with acid.

Vegetation water obtained as described above provides a solution which is rich in low molecular weight polyphenols, particularly hydroxytyrosol and a small amount of tyrosol. The concentration of hydroxytyrosol in the processed water may range from 4 - 5 grams per liter to 10 – 15 grams per liter depending upon 10 the degree of dilution during the olive oil extraction. In one embodiment, the invention provides a method of extraction or purification that selectively enriches the content of hydroxytyrosol without the addition of contaminants. Thus, the major polyphenolic component, hydroxytyrosol, is isolated from other members of the polyphenolic family, impurities, suspended solids, tannins, and other 15 molecules contained in the vegetation water. Hydroxytyrosol may therefore be produced in a purity and quantity not readily available by current synthetic or natural extraction methods.

### B. Treatment of AIDS-Associated Neurological Conditions

20 In accordance with the present invention, there are provided therapeutic methods for treating a variety of conditions related to nervous system disorders.

In one aspect, the invention method comprises administering to a subject in need thereof an effective amount of a treatment agent having a weight ratio of hydroxytyrosol to oleuropein of between about 1:1 and about 200:1.

25 In one embodiment of the invention, the treatment agent is targeted against neurological conditions associated with AIDS. The neurological conditions contemplated for treatment are, in one embodiment, direct or indirect effects of the HIV virus itself. Alternatively, the neurological conditions treated according to the method of the invention are related to opportunistic infections of the nervous 30 system. Preferred AIDS-associated neurological conditions for treatment are as follows:

#### 1. ADC

HIV-1 Associated Dementia, also known as AIDS Dementia Complex (ADC), HIV/AIDS encephalopathy or encephalitis, subacute encephalitis, progressive dementia, HIV/AIDS Related Brain Impairment and AIDS Related Dementia, is a clinical diagnosis based on signs and symptoms of cognitive decline and fine motor dysfunction in the setting of HIV-1 infection, after exclusion of other etiologies. ADC may be progressive or relatively static. In the era of highly-active antiretroviral therapy (HAART), the incidence of fulminant ADC has declined; however, the incidence of a more insidious and protracted dementia, with higher CD4 counts, appears to be increasing (Dore, 1999).

Despite more than a decade of intensive research, the precise pathophysiology of ADC remains elusive. On pathology, atrophy and white matter pallor is observed usually without frank demyelination. Despite widespread scarring (gliosis) and neuronal cell loss, HIV-1 does not directly infect neuronal cells. HIV-1 is found only in macrophages in the brain, and degree of macrophage infiltration, rather than brain viral load, corresponds best to dementia severity (Glass, 1995). Radiographically, the diagnosis is supported by characteristic changes in subcortical and periventricular white matter ("HIV encephalitis"), as well as global cerebral atrophy.

Hence, ADC is considered an "indirect" effect of HIV infection: nervous system injury is driven primarily by the neuroimmunologic host response (Nottet, 1995). While some viral products are neurotoxic in brain cell cultures, it appears that proinflammatory molecules, secreted by or induced by macrophages, are the main effectors of ongoing brain injury. Toxic synergies among viral protein products (viz. Tat) and macrophage-derived cytokines (viz. TNF- $\alpha$ ), interleukins (viz. IL-6), and oxygen free-radical reactions (viz. peroxynitrite) appear to lead to enhanced vulnerability to oxidative stress in the AIDS brain, resulting in massive cell death (Shi, 1998; Lipton, 1995).

There is no approved treatment for ADC. Cognitive improvement may be observed with aggressive antiretroviral therapy in some treatment naïve, or under treated, patients with ADC (Cohen, 2001). Pathologic observations indicate that HIV encephalitis remains a common finding (Masliah, 2000). HAART regimens, while perhaps attenuating the severity of dementia, do not appear to prevent HIV-associated brain injury.

The neurological symptoms associated with ADC have been treated with

certain drugs that have a number of shortcomings. For example, the psychosis associated with HIV dementia has been treated with haloperidol and thioridazine. Molindone has been used for psychotic and delirious HIV dementia patients.

Methylphenidate has been used for treatment of depression associated with ADC.

5      Electro-convulsive therapy has been used for HIV-induced stupor. All of these treatments serve to ameliorate symptoms of ADC. None treat ADC itself.

The conditions treated with the treatment agents include, according to one embodiment of the invention, ADC and the various symptoms with which ADC is associated. An exemplary method of treating ADC is described in Examples 1-3.

10     The treatment agent formulations may be administered to achieve a therapeutic effect and slow or counteract the progression of ADC or they can be administered prophylactically to patients not yet exhibiting ADC but exposed to the HIV virus.

## 2. HIV-Associated Myelopathy

15     HIV-associated myelopathy occurs in approximately 20% of patients with late stage AIDS and is clinically characterized by progressive spasticity and loss of proprioception, predominantly in the lower extremities. Pathologically these findings correlate with vacuolar changes that are most prominent in the posterior and lateral columns of the thoracic spinal cord (termed vacuolar myelopathy) and are

20     morphologically similar to those seen in vitamin B<sub>12</sub> deficiency (Petito et al, 1985). Autopsy studies have shown that vacuolar myelopathy is found in up to 50% of patients dying with AIDS, with only the more severe cases showing symptoms during life (Dal Pan, 1994).

25     Thus, according to one embodiment of the invention, HIV-associated myelopathy and the symptoms with which HIV-associated myelopathy is associated are treated.

## 3. Peripheral Neuropathy

According to another embodiment, this invention provides a therapeutic method for treating a patient suffering from peripheral neuropathy. This method involves administering to the patient an effective peripheral neuropathy-treating amount of one or more of the treating agents or pharmaceutical compositions described. In yet another embodiment, this invention provides a prophylactic method for protecting a patient susceptible to peripheral neuropathy. This

method involves administering to the patient an effective peripheral neuropathy prophylactic amount of one or more of the pharmaceutical compositions or treating agents of the invention.

Peripheral neuropathy is a very common and disabling problem  
5 encountered in HIV infection. It develops primarily in relatively advanced patients with low CD4 counts, and may be exacerbated by the neurotoxicity of several of the drugs commonly used to treat HIV including DDC, DDI, and D4T. However, it is clear that the viral infection itself results in a typical symmetric, painful, distal sensory neuropathy. This entity almost always presents with variable loss of  
10 sensation in the feet and a variety of uncomfortable sensations of swelling, prickling, throbbing or other painful sensations in the feet. This may extend up the legs as it worsens and may eventually start to effect the hands. It occurs in around 20% of AIDS patients, and similar symptoms occur in an even greater number when the drug induced neuropathy is included.

15 Treatment of neuropathic pain such as is encountered in neuropathy is notoriously difficult. Minimizing neurotoxic drugs, optimizing diet, assuring that there are no contributing vitamin deficiencies (especially B<sub>12</sub> and thiamine) are important first steps. Alcohol is often a neurotoxin, and continued heavy alcohol use may worsen symptoms. Routine analgesics such as aspirin and ibuprofen  
20 generally provide little relief. Even narcotics may not fully relieve this kind of pain.

#### 4. Cytomegalovirus encephalitis and radiculomyelitis

Cytomegalovirus is a frequent secondary viral infection in AIDS patients, causing retinitis in up to 40%. Autopsy studies indicate that as many as  
25 20-30% of AIDS patients have CMV encephalitis pathologically, while probably almost 10% develop a clinical neurologic deterioration that is probably the result of CMV. There are two general neurologic syndromes which may occur separately, or in conjunction with each other. The first is the result of CMV attacking the spinal roots and cord resulting in a rapid loss of function of bladder,  
30 saddle anesthesia and legs weakness with variable degree of pain and paralysis. The CSF typically has an inflammatory pattern, and sometimes CMV can be cultured from the CSF. Evaluation of the CSF for CMV DNA reveals abundant viral DNA.

The other presentation may mimic a more aggressive form of AIDS

dementia complex, with symptoms of dementia developing over just a few weeks time, sometimes associated with cranial nerve abnormalities affecting vision, hearing and balance that would be unusual for HIV alone. The spinal fluid is often bland in this disorder, but CSF PCR for CMV DNA is positive, and strongly 5 supports the diagnosis when such a clinical pattern is seen. This disorder is generally rapidly fatal over a period of just 4-8 weeks. Thus, the conditions treated with the treatment agents and pharmaceutical compositions of the invention include, according to one embodiment, CMV encephalitis and radiculomyelitis.

10

#### 5. Progressive Multifocal Leukoencephalopathy (PML)

PML is a lethal secondary viral infection mostly occurring in AIDS patients with advanced immunodeficiency. At present, approximately 6% of AIDS patients die with this condition. The JC virus is a ubiquitous virus, able to enter 15 the brain and lytically infect oligodendrocytes (the cells making myelin in the brain). Thus, demyelination of the brain results, causing a wide variety of focal neurologic symptoms including weakness, loss of sensation, visual loss, changes in balance and coordination. Because it is usually relentlessly progressive, severe neurologic disability develops over a period of 2-6 months with death 20 following rapidly from general disability. According to one embodiment of the invention PML is a condition treated with the treatment agents or pharmaceutical compositions described.

25

#### 6. Other Neurological Diseases

Additional neurological diseases and disturbances contemplated for treatment by the method of the invention include, but are not limited to, Alzheimer's disease; Parkinson's disease; motor neuron diseases such as amyotrophic lateral sclerosis (ALS), Huntington's disease and syringomyelia; ataxias, dementias; chorea; dystonia; dyskinesia; encephalomyopathy; 30 parenchymatous cerebellar degeneration; Kennedy disease; Down syndrome; progressive supernuclear palsy; DRPLA, stroke or other ischemic injuries; thoracic outlet syndrome, trauma; electrical brain injuries; decompression brain injuries; multiple sclerosis; epilepsy; concussive or penetrating injuries of the brain or spinal cord; brain injuries due to exposure of military hazards such as blast

over-pressure, ionizing radiation, and genetic neurological conditions.

By "genetic neurological condition" is meant a neurological condition, or a predisposition to it, that is caused at least in part by or correlated with a specific gene or mutation within that gene; for example, a genetic neurological condition can be caused by or correlated with more than one specific gene. Examples of genetic neurological conditions include, but are not limited to, Alzheimer's disease, Huntington's disease, spinal and bulbar muscular atrophy, fragile X syndrome, FRAXE mental retardation, myotonic dystrophy, spinocerebellar ataxia type 1, dentatorubral-pallidoluysian atrophy, and Machado-Joseph disease.

As described above, presently preferred conditions for treatment in accordance with the present invention include AIDS-associated neurological disorders. An especially preferred condition for treatment in accordance with the present invention includes ADC.

The present invention also relates to combinational therapeutic methods for treating AIDS and AIDS-associated neurological disorders. Combinations of agents contemplated for use in the practice of the present invention are administered to a host in need of such treatment by employing an effective amount of a combination of at least one treating agent useful for the treatment of infectious viral conditions, and at least one AIDS-associated neurological disorder treatment agent as described above. Exemplary HIV antivirals with good CNS penetration include zidovudine, D4T, nevirapine, and abacavir.

### C. Oxidative Stress

Use of the treatment agents or pharmaceutical compositions of the invention, according to one aspect, in preparations to be administrated via different carriers will protect the body against oxidative stress and prevent the outburst of different diseases caused by such oxidative stress.

It is known that free radicals (oxidative stress) of different types are associated with a range of diseases such as ischemic or reperfusion injury, thrombosis and embolism, atherosclerosis, allergic/inflammatory conditions such as bronchial asthma and rheumatoid arthritis, diseases caused by ionizing radiation or ultra violet light, conditions related to neurodegenerative diseases for instance Parkinson's disease and Alzheimer's disease, ageing, apoptosis,

necrosis and cirrhosis, cataract, physical stress, diabetes, autoimmune diseases, intoxications, colitis, hematocrosis, neoplasms and toxicity of antineoplastic or immuno suppressive agents diseases, premature aging or consequences of viral or bacterial infections and endogeneous or exogeneous chemicals present in air, 5 food, general environmental contamination or lifestyle related exposure. Lipid peroxidation or DNA-oxidation caused by excess generation of radicals can constitute significant damaging pathways in the above conditions and diseases.

Oxidative stress can be chemically, physically or biologically induced. 10 Chemically induced oxidative stress is caused by a compound which gives rise to a tissue damage. Physically induced stress is caused by e.g. 1) radiation, such as radioactive or ionizing radiation or UV radiation; 2) by physical blockage of blood flow; biologically induced oxidative stress is the defence by the body itself, with over-reaction of oxidases in phagocytes, extra and intra cellular, one example is HIV patients. Other examples are asthma, rheumatoid arthritis, 15 diabetes etc, cf. above.

Different conditions such as inflammations, infections, gamma-radiation, UV radiation and deficiency of vitamins/antioxidants give rise to oxidative stress which leads to the different conditions and diseases stated above.

Thus, the invention includes, in one aspect, a method of treating oxidative 20 stress in a person in need thereof, comprising administering to said subject a pharmaceutically effective amount of substantially purified hydroxytyrosol or a substantially purified mixture of hydroxytyrosol and oleuropein. The oxidative stress condition may be selected from the group consisting of an inflammatory response, coronary heart disease, and AIDS-associated neurological disorders.

25

#### D. Biological Testing

The effectiveness of a given phenol in treating an AIDS-associated neurological condition may be determined by methods known in the art.

30

##### 1. Neuropsychological Testing

Cognitive evaluation of subjects may be determined by methods known in the art. For example, cognitive evaluation of HIV-1 infected patients may be performed using the tests as previously described by Ryan et al. (2001). Briefly, these tests assess member (Rey Auditory Learning Test), psychomotor speed

(Trail-making), gross and fine motor functioning (Symbol Digit Substitutions, Grooved Pegboard), abstract thinking (Stroop Coor Interference), and mood (Center for Epidemiological Studies Depression Scale, Profile of Mood States).

5 The results of the tests may be analyzed to create a Z score by comparing each patient's performance to a mean score based on education- and age-adjusted normative data. An exemplary neuropsychological test is the NPZ-8 test utilized in Example 1 below.

## 2. Macrophage Activation

10 HIV is found in macrophages in the brain, and degree of macrophage infiltration corresponds well to dementia severity (Adamson, 1999; Glass, 1995). Blood-borne macrophages and/or CD4+ T lymphocytes can carry the virus into the brain and transport it to perivascular and parenchymal blood-derived macrophages and microglia (Bossi, 1998; Ellis, 1997; Brew, 1996).

15 Surrogate markers of macrophage activation in serum and/or CSF may be identified by methods known in the art.

## 3. HIV-1 Viral Load

20 In one embodiment of the invention, the pharmaceutical composition of the invention is administered to HIV-infected patients in amounts and for a time sufficient to induce a clinically significant decrease in HIV viral load. HIV viral load may be measured by any method known to those of skill in the art. An exemplary method of measuring HIV viral load is by determining the level of HIV-RNA (measured in copies per ml) detectable by PCR in the plasma, serum, and/or CSF of an HIV-infected patient, as described in U.S. Patent No. 6,309,632.

## 4. Additional Tests

25 In one embodiment of the invention, neural cell culture systems or *in vivo* biological tests may be used for determining the efficacy of the compositions in reversing neurotoxicity which mimics that observed with ADC, as described in International Patent Application WO 97/38684.

Additional tests contemplated by the invention include: measurement of isoprostane F2, a marker of oxidative stress and lipid peroxidate; plasma cholesterol and HDL; and CSF concentration of mono and polyphenol metabolites. Methods for

performing these tests are known to those of skill in the art.

**E. Treatment of Inflammation or Inflammation-associated Conditions**

As noted above, in accordance with another aspect of the invention, there  
5 are provided therapeutic methods for treating a variety of conditions related to  
inflammation or inflammation-associated disorders.

One role of the inflammatory response serves the purpose of eliminating  
harmful molecules from the body. A wide range of pathogenic insults can initiate  
an inflammatory response. These include autoimmune stimuli, infections,  
10 allergens, immune response to transplanted tissue, noxious chemicals, toxins,  
ischemia/reperfusion, hypoxia, and mechanical and thermal trauma. Typically,  
inflammation is a very localized response that serves to expulse, attenuate by  
dilution, and isolate the damaging agent and injured tissue. The body's response  
becomes an agent of disease when it results in inappropriate injury to host  
15 tissues in the process of eliminating the targeted agent, or responding to a  
traumatic insult.

Inflammation is a component of pathogenesis in a number of vascular  
diseases or injuries, including atherosclerosis. Blake, GJ and Ridker, PM (2002)  
*J Internal Medicine* 252:283-294. The cells involved with inflammation include  
20 leukocytes (i.e., the immune system cells - neutrophils, eosinophils, lymphocytes,  
monocytes, basophils, macrophages, dendritic cells, and mast cells), the vascular  
endothelium, fibroblasts, vascular smooth muscle cells, and myocytes.

The release of inflammatory cytokines such as tumor necrosis factor-alpha  
(TNF- $\alpha$ ) by leukocytes is a means by which the immune system combats  
25 pathogenic invasions, including infections. TNF- $\alpha$  stimulates the expression and  
activation of adherence factors on leukocytes and endothelial cells, primes  
neutrophils for an enhanced inflammatory response to secondary stimuli and  
enhances adherent neutrophil oxidative activity. In addition,  
macrophages/dendritic cells act as accessory cells processing antigen for  
30 presentation to lymphocytes. The lymphocytes, in turn, become stimulated to act  
as pro-inflammatory cytotoxic cells.

Generally, cytokines stimulate neutrophils to enhance oxidative and  
nonoxidative inflammatory activity. Inappropriate and over-release of cytokines  
can produce counterproductive exaggerated pathogenic effects through the

release of tissue-damaging oxidative and nonoxidative products. For example, TNF $\alpha$  can induce neutrophils to adhere to the blood vessel wall and then to migrate through the vessel to the site of injury and release their oxidative and non-oxidative inflammatory products.

5

Thus, the method of the present invention includes, in one aspect, a method of treating an inflammatory condition in a subject in need of such treatment. The method includes administering to the subject a pharmaceutically effective amount of substantially purified hydroxytyrosol or a substantially purified mixture of hydroxytyrosol and oleuropein.

10 The subject may have an inflammatory condition characterized by a detectable clinical symptom or change in a level of a biochemical marker with respect to the normal range of the marker.

15 A dose of an olive plant extract treatment agent having a weight ratio of hydroxytyrosol to oleuropein of between about 1:1 and about 200:1 is administered to the subject. Preferably, the weight ratio is between about 5:1 and about 100:1. More preferably, the weight ratio is between about 10:1 and about 50:1. The administration is continued until there is observed a return of the marker level to the normal range, a desired, measurable decrease in the level of 20 the marker, or a desired change in the clinical symptom.

25 The marker or the clinical symptom may include any number of markers or clinical symptoms which are generally known in the art to be associated with inflammation. Preferably, the symptoms and markers are associated with specific types of inflammation. These include (i) the symptoms and markers in joint pain and swelling in the case of joint inflammation; (ii) elevated levels of C-reactive protein in the case of coronary inflammation; (iii) respiratory distress in the case of bronchial inflammation; and (iv) elevated CSF levels of isoprostanes or functional or psychofunctional indicators in the case of neuro-inflammation. The marker may be a cytokine, such as TNF- $\alpha$ , interleukin-1, interleukin-6, and/or interleukin-30 8. Other markers include corticotrophin, cortisol and/or prolactin.

In the case of joint inflammation, the inflammatory condition may be rheumatoid arthritis, osteoarthritis, and/or other inflammatory conditions involving acute joint inflammation, chronic joint inflammation, or both.

Additional inflammatory conditions that may be treated with the agent or

extract of the invention include conditions such as delayed type hypersensitivity reaction, a symptom of psoriasis, an autoimmune disease, organ transplant, pain, fever, or tissue graft rejection. Exemplary autoimmune diseases are Reynaud's syndrome, autoimmune thyroiditis, EAE, multiple sclerosis or lupus erythematosus.

5 Additional exemplary inflammatory conditions include adult respiratory distress syndrome (ARDS), multiple organ injury syndromes secondary to septicemia or trauma, reperfusion injury of myocardial or other tissues, acute glomerulonephritis, reactive arthritis, dermatoses with acute inflammatory 10 components, acute purulent meningitis or other central nervous system inflammatory disorders, thermal injury, hemodialysis, leukapheresis, ulcerative colitis, Crohn's disease, necrotizing enterocolitis, granulocyte transfusion associated syndromes, cytokine-induced toxicity, asthma, psoriasis, skin sunburn, inflammatory pelvic disease, inflammatory bowel disease, urethritis, uritis, 15 senusitis, pneumonitis, encephalitis, meningitis, myocarditis, nephritis, osteomyelitis, myositis, hepatitis, gastritis, enteritis, dermatitis, gingivitis, appendicitis, pancreatitis, cholecystitis and cholangitis.

The inflammatory condition may be an acute inflammatory reaction or an allergic inflammatory reaction.

20 The treatment agent mixture may be prepared by any number of methods known in the art. Preferably, the treatment agent is an extract prepared by the process described in Section II.A. above. A particularly preferable method of preparing the treatment agent involves producing vegetation water from olives; optionally adding acid to the vegetation water in an amount effective to produce a 25 pH between about 1 and about 5; and incubating the acidified vegetation water until a substantial portion, e.g. at least 75%, of oleuropein originally present in the vegetation water has been converted to hydroxytyrosol.

30 A wide variety of administration methods are contemplated by the present invention and discussed in further detail in Section III below. These include oral delivery, intramuscular injection, intravenous injection, transdermal delivery, and transmucosal delivery. Oral delivery is preferred.

The compounds of this invention are useful in the treatment or alleviation of inflammation, other inflammation associated disorders, such as arthritis, neurodegeneration and colon cancer, in mammals, preferably humans, dogs, cats

or livestock animals.

The amount of therapeutically active compound that is administered and the dosage regimen for treating a disease condition with the compounds and/or composition of this invention depends on a variety of factors, including the age, 5 weight, sex and medical condition of the subject, the severity of the disease, the route and frequency of administration, and the particular compound employed, and thus may vary widely. The pharmaceutical compositions may contain active ingredient in the range of about 0.1 to 2000 mg/kg body weight, preferably in the range of about 0.3 to 50 mg/kg and most preferably about 0.6mg/kg. A daily dose 10 can be administered in one to four doses per day. In one embodiment a daily dose of 20mg is delivered in 4 doses of 5mg each.

The active agent or extract of the present invention may be administered in combination with other agents. The agents may be administered before, after, and/or during administration with the primary treatment agent. Preferable 15 combinatorial agents include glucosamine sulfate, chondroitin sulfate, sea cucumber extract, hydrolyzed shark cartilage, collagen II, and methylsulfonylmethane.

Additional inhibitors of mediators of inflammation contemplated for use in combination with the active agent or extract of the present invention include 20 matrix metalloproteinase inhibitors, aggrecanase inhibitors, TACE inhibitors, leukotriene receptor antagonists, IL-1 processing and release inhibitors, IL-1 ra, H (1 )-receptor antagonists; kinin-B (1 )- and B (2 )- receptor antagonists; prostaglandin inhibitors such as PGD-, PGF- PGI ( 2 ), and PGE-receptor antagonists; thromboxane A (2 )(TXA2-) inhibitors; 5- and 12-lipoxygenase 25 inhibitors; leukotriene LTC (4 )-, LTD (4 )/LTE (4 )-, and LTB (4 )-inhibitors; PAF-receptor antagonists; gold in the form of an aurothio group together with various hydrophilic groups; immunosuppressive agents, e.g., cyclosporine, azathioprine, and methotrexate; anti-inflammatory glucocorticoids; penicillamine; hydroxychloroquine; anti-gout agents, e.g., colchicine, xanthine oxidase inhibitors, 30 e.g., allopurinol, and uricosuric agents, e. g., probenecid, sulfinpyrazone, and benzboromarone.

### III. Pharmaceutical Compositions

The therapeutic compositions described herein may be administered

subcutaneously, intravenously, parenterally, intraperitoneally, intradermally, intramuscularly, topically, enteral (for example, orally), rectally, nasally, buccally, vaginally, by inhalation spray, by drug pump or via an implanted reservoir in dosage formulations containing conventional nontoxic, physiologically (or 5 pharmaceutically) acceptable carriers or vehicles.

In a specific embodiment, it may be desirable to administer the agents of the invention locally to a localized area in need of treatment; this may be achieved by, for example, and not by way of limitation, local infusion during surgery, topical application, transdermal patches, by injection, by means of a 10 catheter, by means of a suppository, or by means of an implant, said implant being of a porous, non-porous, or gelatinous material, including membranes; such as sialastic membranes or fibers.

When it is desirable to direct the drug to the central nervous system, techniques which can opportunistically open the blood brain barrier for a time 15 adequate to deliver the drug there through can be used. For example, a composition of 5% mannitose and water can be used. The present invention also provides pharmaceutical compositions. Such compositions comprise a therapeutically (or prophylactically) effective amount of the agent, and a physiologically acceptable carrier or excipient. Such a carrier includes, but is not 20 limited to, saline, buffered saline, dextrose, water, glycerol, ethanol, and combinations thereof. The carrier and composition can be sterile. The formulation should suit the mode of administration.

Suitable pharmaceutically acceptable carriers include but are not limited to water, salt solutions (for example, NaCl), alcohols, gum arabic, vegetable oils, 25 benzyl alcohols, polyethylene glycols, gelatin, carbohydrates such as lactose, amylose or starch, magnesium stearate, talc, silicic acid, viscous paraffin, perfume oil, fatty acid esters, hydroxymethylcellulose, polyvinyl pyrrolidone, etc. The pharmaceutical preparations can be sterilized and if desired, mixed with auxiliary agents, for example, lubricants, preservatives, stabilizers, wetting 30 agents, emulsifiers, salts for influencing osmotic pressure, buffers, coloring, flavoring and/or aromatic substances and the like which do not deleteriously react with the active compounds.

The compositions, if desired, can also contain minor amounts of wetting or emulsifying agents, or pH buffering agents. The composition can be a liquid

solution, suspension, emulsion, tablet, pill, capsule, sustained release formulation, or powder. The composition can be formulated as a suppository, with traditional binders and carriers such as triglycerides. Oral formulation can include standard carriers such as pharmaceutical grades of mannitol, lactose, 5 starch, magnesium stearate, polyvinyl pyrrolidone, sodium saccharine, cellulose, magnesium carbonate, etc.

The compositions can be formulated in accordance with the routine procedure as a pharmaceutical composition adapted for intravenous administration to human beings. Typically, compositions for intravenous 10 administration are solutions in sterile isotonic aqueous buffer. Where necessary, the composition may also include a solubilizing agent and a local anesthetic to ease pain at the site of the injection.

Generally, the ingredients are supplied either separately or mixed together in unit dosage form, for example, as a dry lyophilized powder or water free 15 concentrate in a hermetically sealed container such as an ampoule or sachette indicating the quantity of active agent. Where the composition is to be administered by infusion, it can be dispensed with an infusion bottle containing sterile pharmaceutical grade water, saline or dextrose/water. Where the 20 composition is administered by injection, an ampule of sterile water for injection or saline can be provided so that the ingredients may be mixed prior to administration.

For topical application, there are employed as nonsprayable forms, viscous to semi-solid or solid forms comprising a carrier compatible with topical application and having a dynamic viscosity preferably greater than water. Suitable 25 formulations include but are not limited to solutions, suspensions, emulsions, creams, ointments, powders, enemas, lotions, sols, liniments, salves, aerosols, etc., which are, if desired, sterilized or mixed with auxiliary agents, for example, preservatives, stabilizers, wetting agents, buffers or salts for influencing osmotic pressure, etc. The drug may be incorporated into a cosmetic formulation. For 30 topical application, also suitable are sprayable aerosol preparations wherein the active ingredient, preferably in combination with a solid or liquid inert carrier material, is packaged in a squeeze bottle or in admixture with a pressurized volatile, normally gaseous propellant, e.g., pressurized air.

The amount of agent or agents which will be effective in the treatment of a

particular disorder or condition will depend on the nature of the disorder or condition, and can be determined by standard clinical techniques. In addition, in vitro or in vivo assays may optionally be employed to help identify optimal dosage ranges. The precise dose to be employed in the formulation will also depend on the route of administration, and the seriousness of the disease or disorder, and should be decided according to the judgment of the practitioner and each patient's circumstances.

From the foregoing, it can be seen how various objects and features of the invention are met.

10

#### IV. Examples

The following examples further illustrate the invention described herein and are in no way intended to limit the scope of the invention.

15

##### Example 1

###### Study Procedures

This is a prospective, randomized, double-blind, placebo-controlled six-week parallel study of up to 32 subjects with HIV-associated cognitive dysfunction who have been on a stable medication regimen (antiretroviral and other medications) for at least 8 weeks. Subjects will be stratified at randomization, based on a "biased coin" paradigm, with regard to HIV-1 Viral Load <50,000 copies/ml versus equal to or greater than 50,000 copies/ml, to insure the two groups are balanced with regard to this important clinical variable. Subjects will receive either the active ingredient (20 mg total phenols) or identical placebo tablets (provided by the manufacturer) to be taken orally. Subject, Investigator and Sponsor will be blinded to treatment assignment until study completion.

At baseline and at Study Week 6, neuropsychiatric testing (NPZ-8), neurological examination, urinary F2 Isoprostane, serum beta-2 microglobulin, HIV-1 Viral Load, CD4 lymphocyte count, serum chemistries (electrolytes, renal and liver function tests), coagulation profile, and lipid profile will be obtained. Approximately 15mL of blood will be obtained at baseline and at 6 weeks from each subject. A urine sample of 20 mL will be obtained at these time points. At study weeks 2 and 4, subjects will be contacted by the study assistant by

telephone with regard to any changes in status or adverse events. Adverse events will be evaluated by the PI as appropriate.

It is anticipated that up to 8 subjects will consent to lumbar puncture at baseline and at 6 weeks for assessment of CSF F2 Isoprostan e, B2 5 microglobulin, CSF viral load, and measurement of Hydroxytyrosol-derived metabolites. LP will be performed by the PI.

The PI, for example, a board-certified neurologist, neuro-AIDS specialist and PI in previous ADC trials, will perform patient selection and neurologic examinations. The neuropsychiatric battery, the NPZ-8, takes about 30 minutes 10 to administer, and has been validated in the ADC population and is the most commonly used instrument in ADC treatment trials. The neurologic examination will take approximately 30 minutes. The baseline and follow-up visits should take no more than 90 minutes each, including phlebotomy, for the average subject. Each follow-up telephone call will take, on average, approximately 10 minutes.

15 Urinary and, if available, CSF F2-isoprostan e assays will be performed at a state-of-the-art laboratory which performs this and other prostaglandin assays. CSF phenol (hydroxytyrosol) concentrations will be measured, blind to treatment assignment of subjects

Subjects willing to undergo baseline and follow-up lumbar puncture will be 20 consented separately. No subject will be denied participation in the study because of reluctance to undergo LP. The procedure typically takes 20 minutes. Subjects can, if they wish, remain supine for a period of time after the procedure. However, contrary to common belief, remaining supine post LP is not associated 25 with reduction in incidence (10%) of post LP headache. Should a post LP headache occur, the subject will be offered treatment (blood patch: >85% success rate).

#### Example 2

#### Data Analysis

30 Safety data will be summarized descriptively for the two groups—active vs. placebo—using COSTART terms for mapping adverse events.

Change scores in laboratory safety evaluations (serum electrolytes, BUN, creatinine, CBC and differential, PT, PTT, platelets, total and HDL cholesterol, AST, ALT, total bilirubin) will be summarized.

The primary efficacy variable—difference in group mean concentrations of Isoprostane F2 at Study Week 6 compared with baseline—will be analyzed using 2-sided student's T test, with normalization of baseline concentrations. Because a normal distribution of this variable cannot be assumed, a Wilcoxon signed rank 5 test will also be used to examine the difference in Iso-F2 from baseline to Study Week 6 in individual subjects, ranked according to magnitude, with determination of the test statistic to compare the treatment versus placebo groups. With regard to secondary variables, these approaches will be used to examine differences from baseline in B2 microglobulin and HIV-1 viral load in serum and, where 10 possible, in urine. Descriptive summary statistics will be provided for subgroups. All analyses will be done by an independent third party using SAS software.

### Example 3

#### Subject Selection

15 All subjects will be HIV-1 seropositive by Elisa, confirmed by Western Blot. Rationale for studying this population: there is no proven treatment for ADC or HIV-associated cognitive motor syndrome. The brain injury that occurs as a result of HIV-1 infection continues to afflict AIDS patients, despite highly-active antiretroviral therapy. As HIV-1 infected individuals live longer, ADC prevalence, 20 and perhaps incidence, will likely rise.

Up to 32 subjects will be enrolled. It is expected that full data will be then available for approximately 12 subjects in each group, taking into account a 20% drop out rate typical from an ADC clinical trial. The sample size is reasonable for a pilot safety and tolerability study. Statistical significance is not expected for the 25 primary efficacy endpoint in this small study; a trend towards group differences may be detected.

HIV-1 seropositive men and women over the age of 18, with signs and 30 symptoms of HIV-associated cognitive-motor syndrome or frank ADC (Stage 0.5-2 Memorial Sloan Kettering AIDS Dementia Scale) for at least 3 months, and a Karnovsky score >50 (able to care for most of daily needs, but may need some assistance) will be eligible for enrollment. Subjects will have been on a stable regimen of antiretroviral and any other medical treatments for at least 3 months, and will reasonably be expected to remain on their current regimen for the duration of the study. Subjects in other investigational protocols or agents will be

excluded. Other causes of cognitive impairment, such as thyroid disease, vitamin deficiency, testosterone deficiency (if male), neurosyphilis, other CNS disease, uncontrolled epilepsy, untreated depression, hepatic or uremic encephalopathy, traumatic brain injury or drug or alcohol abuse will be excluded per standard evaluation prior to enrollment. If an appropriate workup has not been performed, the potential subject will be referred back to his or her primary provider for evaluation. Subjects will be ambulatory, and have no active clinically significant systemic disease (other than HIV) that would, in the PI's judgment, preclude safe participation or compliance with the protocol.

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Initial results from the above examples 1-3 showed a statistically significant favorable change in 8-isoprostane levels in the urine.

#### Example 4

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##### Arthritis Studies

Test a group of individuals with Rheumatoid Arthritis and a group with Osteoarthritis with the stress reactivity protocol, before and after 4 weeks of active agent (20mg total phenols) supplementation and compare to controls over the same time period with no supplementation. Measurements will include heart rate, 20 blood pressure, cortisol, ACTH, PRL, cytokines, and CRP.

Test individuals with Rheumatoid Arthritis, before and after 10 weeks of active agent supplementation and compare to a group doing water aerobic exercise and a control group that does no intervention. Measurements will include assessment of disease activity, prostaglandins, cortisol, PRL, CRP, mood changes, fitness measures, and endurance.

Test individuals with Rheumatoid Arthritis, with and without active agent supplementation for four weeks, and examine their acute phase response to an exercise strength test using a leg press set at 70% of the 1Rep Max. Measurements will include cortisol, PRL, CRP, and a panel of inflammatory 30 cytokines.

Evaluate increasing concentrations of active agent in a collagen-induced arthritis mouse model at 1.3mg, 13mg and 130mg/mouse; with and without Cox-2 inhibitors. The positive control involves treatment with Cox-2 inhibitors, NSAID, or steroids at the highest dose levels. Parameters such as swelling determined by

ankle width measurement; histopathological score involving subsynovial inflammation characterized by mononuclear cell resembling nodule formation, pannus formation, bone erosion and synovial hyperplasia; radiological score; and cytokine analysis score measuring levels of TNF- $\alpha$ , IL-1, JE/CCLE (mouse

5 Monococyte Chemotactic Protein-1), and IL-8.

Use of the mouse model as an acute inflammation/injury model to evaluate inhibitors that increase survival by reducing the levels of TNF- $\alpha$  and IL-1 induced by a single intraperitoneal injection of 50 $\mu$ g of LPS and D-Galactosamine (600mg/kg). In this model, mice are treated with the active agent (1.3; 13; and 10 130mg/mouse) 12 hours prior to injection with LPS/D-Gal i.p. Survival of mice is followed for 72 hours following injection with LPS/D-Gal. At 72 hours, blood is collected from all mice by retro-orbital puncture for measurement of TNF- $\alpha$  and IL-1 levels by ELISA.

Additional exemplary studies include TNF- $\alpha$  and IL-1 production induced by LPS by THP-1 monocytes, IL-8 production by fibroblasts; the effects of the active agent on the levels of superoxide and hydrogen peroxide production from human neutrophils; and basal levels of Cox-1 and LPS-induced levels of Cox-2 regulation in monocytes and fibroblasts with active agent added prior to LPS addition.

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All publications and patent applications mentioned in this specification are indicative of the level of skill of those skilled in the art to which this invention pertains. All publications and patent applications are herein incorporated by reference to the same extent as if each individual publication or patent application was specifically and individually indicated to be incorporated by reference.

25 Although the invention has been described with respect to particular embodiments, it will be apparent to those skilled in the art that various changes and modifications can be made without departing from the invention.

## IT IS CLAIMED:

1. A method of treating an AIDS-associated neurological disorder in a subject in need of such treatment, comprising administering to said subject a pharmaceutically effective amount of a treatment agent having a weight ratio of hydroxytyrosol to oleuropein of between about 1:1 and about 200:1.
2. The method of claim 1, wherein said weight ratio is between about 5:1 and about 100:1.
3. The method of claim 2, wherein said weight ratio is between about 10:1 and about 50:1.
4. The method of claim 2, wherein said agent is prepared by a process comprising the steps of:
  - (a) producing vegetation water from olives;
  - (b) adding acid to the vegetation water in an amount effective to produce a pH between about 1 and about 5;
  - (c) incubating the acidified vegetation water until at least 75% of oleuropein originally present in the vegetation water has been converted to hydroxytyrosol.
5. The method of claim 1, wherein said disorder is AIDS dementia.
6. The method of claim 1, wherein said administering comprises oral delivery.
7. The method of claim 1, wherein said administering further comprises administering a second disease treatment agent.
8. The method of claim 7, wherein said administering of the second treatment agent is before or after administration of the first treatment agent.
9. The method of claim 7, wherein said administering of a second treatment agent is coincident with administering the first treatment agent.

10. The method of claim 7, wherein said second agent is an antiretroviral agent.

5 11. The method of claim 1, wherein the agent is administered at a dosage of between about 0.1 mg/kg and 2000 mg/kg per day.

12. The method of claim 11, wherein the agent is administered at a dosage of between about 0.3 mg/kg and 1 mg/kg per day.

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13. The method of claim 12, wherein the agent is administered at a dosage of about 0.6 mg/kg per day.

14. The method of claim 1, wherein said subject is a human.

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15. The method of claim 1, wherein said agent is dried to provide a powder extract.

20 16. The method of claim 1, wherein said agent is in the form of a tablet, capsule, or pill.

17. The method of claim 1, wherein said agent is in the form of a liquid (drops).

25 18. A method of treating an AIDS-associated neurological disorder in a subject in need of such treatment, comprising administering to said subject a pharmaceutically effective amount of substantially purified hydroxytyrosol or a substantially purified mixture of hydroxytyrosol and oleuropein.

30 19. A method of treating a subject having an inflammatory condition characterized by a detectable clinical symptom or change in a level of a biochemical marker with respect to the normal range of the marker, the method comprising

administering to the subject a dose of an olive plant extract treatment

agent having a weight ratio of hydroxytyrosol to oleuropein of between about 1:1 and about 200:1; and

continuing said administration until there is observed a return of the marker level to the normal range or a desired change in the clinical symptom,

5 where the marker or the clinical symptom is selected from the group consisting of

(i) the symptoms and markers in joint pain and swelling in the case of joint inflammation;

10 (ii) elevated levels of C-reactive protein in the case of coronary inflammation;

(iii) respiratory distress in the case of bronchial inflammation; and

(iv) elevated CSF levels of isoprostanes or functional or psychofunctional indicators in the case of neuro-inflammation.

15 20. The method of claim 19, wherein the marker is a cytokine selected from the group consisting of tumor necrosis factor- $\alpha$ , interleukin-1, interleukin-6, and interleukin-8.

20 21. The method of claim 19, wherein the marker is selected from the group consisting of corticotrophin, cortisol and prolactin.

25 22. The method of claim 19, wherein said weight ratio is between about 5:1 and about 100:1.

23. The method of claim 22, wherein said weight ratio is between about 10:1 and about 50:1.

30 24. The method, according to claim 19, wherein said inflammatory condition is selected from the group consisting of rheumatoid arthritis, osteoarthritis, and other inflammatory conditions involving acute joint inflammation, chronic joint inflammation, or both.

25. The method of claim 19, wherein said extract is prepared by a process comprising the steps of:

- (a) producing vegetation water from olives;
- (b) adding acid to the vegetation water in an amount effective to produce a pH between about 1 and about 5;
- (c) incubating the acidified vegetation water until at least 75% of oleuropein originally present in the vegetation water has been converted to hydroxytyrosol.

5 26. The method of claim 19, wherein said administering comprises a method selected from the group consisting of oral delivery, intramuscular injection, intravenous injection, transdermal delivery, and transmucosal delivery.

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27. The method of claim 26, wherein said administering comprises oral delivery.

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28. The method of claim 19, wherein said administering further comprises administering a second disease treatment agent.

29. The method of claim 28, wherein said administering of the second treatment agent is before or after administration of the first treatment agent.

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30. The method of claim 28, wherein said administering of a second treatment agent is coincident with administering the first treatment agent.

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31. The method of claim 28, wherein the second treatment agent comprises one or more of the components selected from the group consisting of glucosamine sulfate, chondroitin sulfate, sea cucumber extract, hydrolyzed shark cartilage, collagen II, and methylsulfonylmethane.

32. The method of claim 19, wherein the agent is administered at a dosage of between about 0.1 mg/kg and 2000 mg/kg per day.

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33. The method of claim 32, wherein the agent is administered at a dosage of between about 0.3 mg/kg and 1 mg/kg per day.

34. The method of claim 33, wherein the agent is administered at a

dosage of about 0.6 mg/kg per day.

35. The method of claim 19, wherein said subject is a human.
- 5 36. The method of claim 19, wherein said agent is dried to provide a powder extract.
- 10 37. The method of claim 19, wherein said agent is in the form of a tablet, capsule, or pill.
38. The method of claim 19, wherein said agent is in the form of a liquid or liquid drops.
- 15 39. A method of treating an inflammatory condition in a subject in need of such treatment, comprising administering to said subject a pharmaceutically effective amount of substantially purified hydroxytyrosol or a substantially purified mixture of hydroxytyrosol and oleuropein.
- 20 40. The method of claim 39, wherein said inflammatory condition is in response to a condition selected from the group consisting of: delayed type hypersensitivity reaction, a symptom of psoriasis, an autoimmune disease, organ transplant, pain, fever, and tissue graft rejection.
- 25 41. The method of claim 40, wherein the autoimmune disease is selected from the group consisting of: Reynaud's syndrome, autoimmune thyroiditis, EAE, multiple sclerosis and lupus erythematosus.
- 30 42. The method of claim 39, wherein the inflammatory condition is in response to a condition selected from the group consisting of: adult respiratory distress syndrome (ARDS), multiple organ injury syndromes secondary to septicemia or trauma, reperfusion injury of myocardial or other tissues, acute glomerulonephritis, reactive arthritis, dermatoses with acute inflammatory components, acute purulent meningitis or other central nervous system inflammatory disorders, thermal injury, hemodialysis, leukapheresis, ulcerative

colitis, Crohn's disease, necrotizing enterocolitis, granulocyte transfusion associated syndromes, and cytokine-induced toxicity.

43. The method of claim 39, wherein said inflammatory condition results from a condition selected from the group consisting of asthma, psoriasis, skin sunburn, inflammatory pelvic disease, inflammatory bowel disease, urethritis, uritis, senusitis, pneumonitis, encephalitis, meningitis, myocarditis, nephritis, osteomyelitis, myositis, hepatitis, gastritis, enteritis, dermatitis, gingivitis, appendicitis, pancreatitis, cholecystitis and cholangitis.

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44. The method of claim 39, wherein the inflammatory condition is an acute inflammatory reaction.

45. The method of claim 39, wherein the inflammatory condition is an allergic inflammatory reaction.

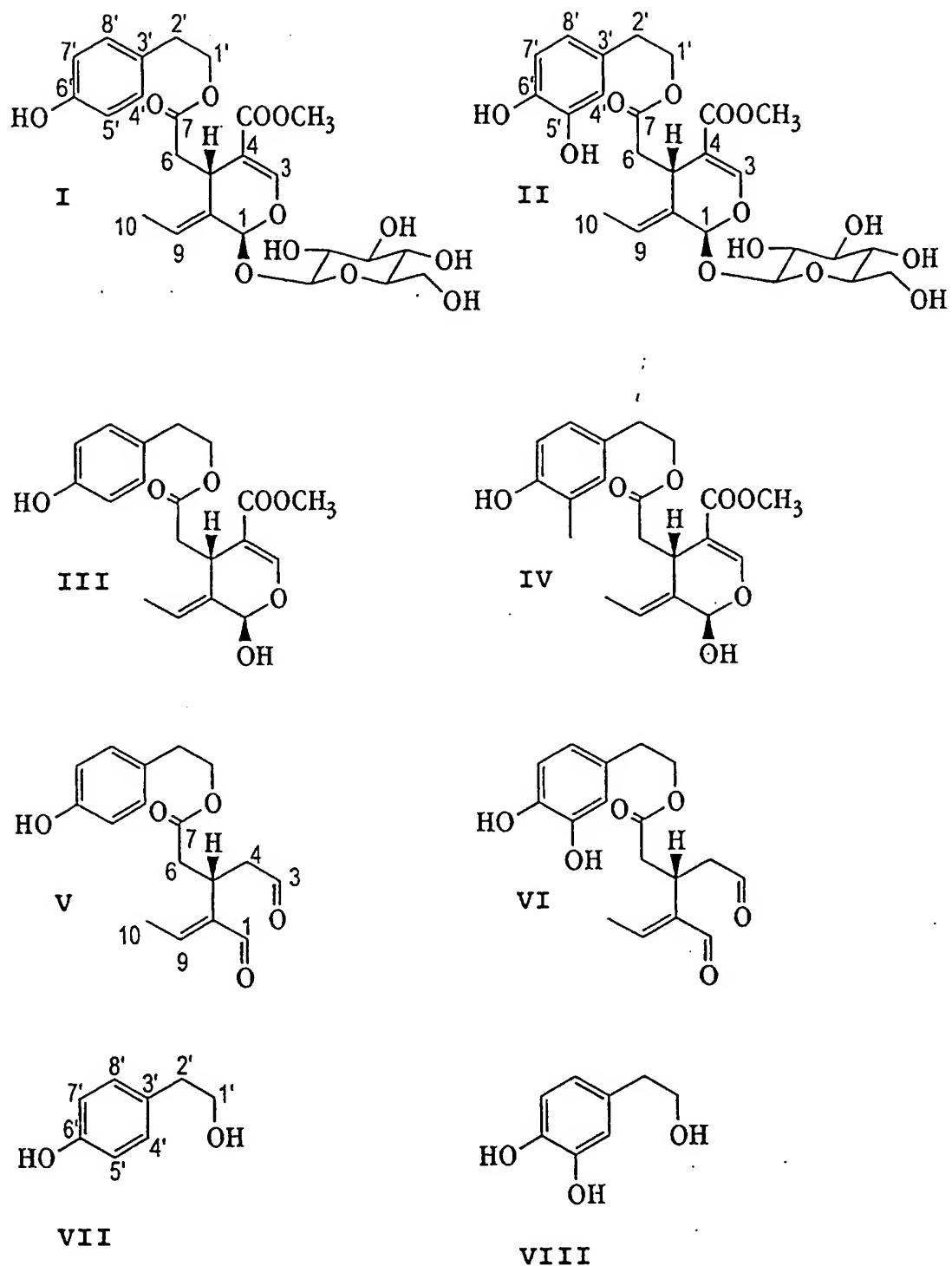


Fig. 1